GRAFT PROCESSING

399

Anti-CD20 Chimeric Antigen Receptor (CAR) Modified Expanded Natural Killer (NK) Cells Significantly Mediate Rituximab Sensitive and Resistant Burkitt Lymphoma (BL) Regression and Improve Survival in Human BL Xenografted NSG Mice

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Background: Burkitt lymphoma (BL) is the most common form of non-Hodgkin lymphoma that occurs in children and adolescents (Miles/Cairo, BJH, 2012). The prognosis for patients with relapsed/resistant BL is very dismal (Cairo, et al, *J Clin Oncol*, 2012). We have previously reported that expanded Peripheral Blood Natural Killer (exPBNK) Cells electroporated with anti-CD20 Chimeric Antigen Receptor (CAR) mRNA have significant cytotoxicity against $CD20^+$ Rituximab (Rx) sensitive and resistant BL *in vitro* (Chu/Cairo, et al, ASH, 2012).

Objective: To examine the anti-tumor effect of anti-CD20 chimeric antigen receptor (CAR^+) modified exPBNK against $CD20^+$ Rx sensitive and resistant BL in vivo using xenografted NSG mice.

Methods: anti-CD20 CAR mRNA was nucleofected into $CD56^+CD3^-$ exPBNK and CAR expression was detected by flow cytometry as described (Chu & Cairo, et al, ASH, 2012).

$5x10^5$ Rx sensitive (Raji) or resistant (Raji-2R) cells expressing luciferase was i.p. or s.c. injected into the 6 wks-old NSG mice. The engraftment and progression were evaluated using the Xenogen IVIS200 system after injection of D-luciferin. After the engraftment was verified, $5x10^6$ CAR exPBNK, MOCK exPBNK (no CAR mRNA electroporation) or medium only was i.p. injected to each mouse once a week for 3 weeks. The cumulative luciferase signals and tumor size were measured weekly to indicate the tumor growth, dissemination and progression. The results with a P value < 0.05 were deemed statistically significant.

Results: In the Raji xenografted mice model, after the third injection, the luciferase signals measured in the CAR exPBNK treated Raji mice were significantly reduced than that in the control mice (P=0.0087) and the mock exPBNK treated mice (P=0.0128) (Fig.1A). And the CAR exPBNK treated Raji mice had significantly extended survival time with median 40 days compared to the untreated mice (29 days, P<0.001) and the mock exPBNK treated mice (30 days, P<0.001) (Fig.1B).

Similarly, in the Raji-2R xenografted mice model, after the third injection, the luciferase signals were also significantly reduced in the CAR exPBNK treated Raji-2R mice compared to the mock exPBNK treated mice (P<0.01). And the tumor size measured in the CAR exPBNK treated Raji-2R mice was significantly smaller than that in the control mice (P=0.0175) and the mock exPBNK treated mice (P=0.0122) (Fig.2A). The CAR exPBNK treated Raji-2R mice had significantly extended survival time with median 24 days compared to the untreated mice (18 days, P<0.001) and the mock exPBNK treated mice (22 days, P<0.05) (Fig.2B).

Conclusion: Multiple injections of anti-CD20 CAR mRNA electroporated exPBNK cells can significantly mediate Rx sensitive and resistant BL tumor regression and extend the survival of BL xenografted mice. These results indicate therapeutic potential of multiple injections of CAR mRNA modified exPBNK cells against relapsed/resistant BL in patients.

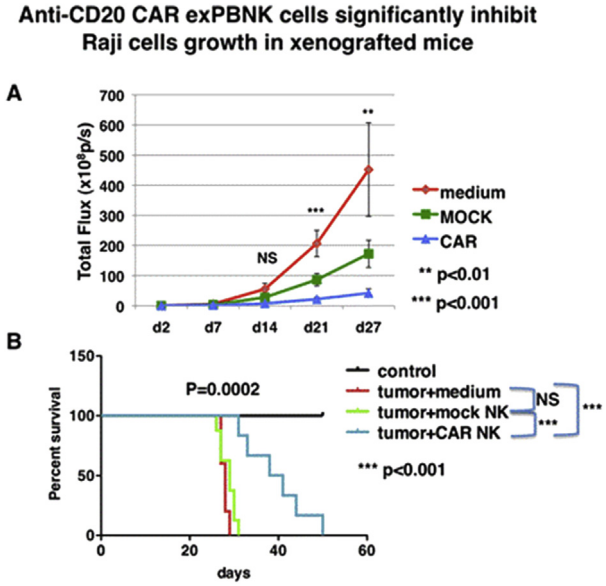

Figure 1.

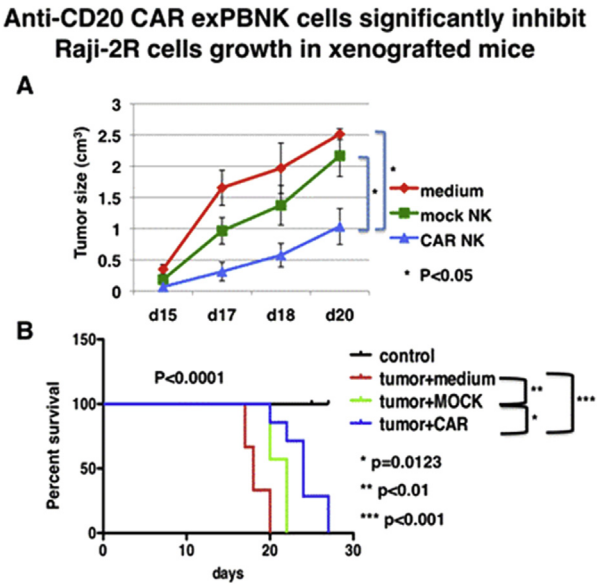

Figure 2.